IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Fastrez et al.
Serial No.	:	08/978,607
Filed	:	November 26, 1997
For	:	***CHIMERIC TARGET MOLECULES HAVING A REGULATABLE ACTIVITY***
Group Art Unit	:	1642
Examiner	:	T. Saidha

I certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:

Assistant Commissioner for Patents
Box RCE
Washington, D. C. 20231, April 2, 2002

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Signature

April 2, 2002
Date of Signature

BOX RCE

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AMENDMENT

Marked up version

Sir:

In response to the Final Office Action mailed March 15, 2000, Applicants respectfully request entry of the following enclosed Request for Continued Examination (RCE) under 37

C.F.R. § 1.114 and the following Amendment. A petition to revive an unintentionally abandoned application, a petition for a three (3) months extension of time from June 15, 2000 to September 15, 2000, a Sequence Listing, and two diskettes in computer readable form containing the Sequence Listing are enclosed.

IN THE CLAIMS:

13. (Twice Amended) A method for determining the presence or amount of an analyte in a test sample, comprising:

forming a mixture of [contacting] (1) a chimeric enzyme comprising an enzyme and a mimetope, said mimetope including at least one amino acid, said chimeric enzyme having a sequence of said mimetope inserted in said enzyme or replacing at least one amino acid of said enzyme with the proviso that the activity of the chimeric enzyme is modulated upon binding of a binding molecule to the mimetope, [with a] ([1] 2) a test sample containing said analyte of interest, ([2] 3) a binding molecule which binds to a mimetope of the chimeric enzyme and modulates the activity of the enzyme, and ([3] 4) a substrate upon which the chimeric enzyme catalytically acts;[, to form a reaction mixture, wherein said chimeric enzyme comprises a starting enzyme comprising a polypeptide and said mimetope, said mimetope comprising at least one amino acid, wherein said chimeric enzyme is constructed by inserting a sequence of said mimetope into a sequence of said starting enzyme or replacing at least one amino acid of the starting enzyme with a sequence of said mimetope, with the proviso that the enzymatic activity of the chimeric enzyme is equivalent to that of the starting enzyme and the activity of the chimeric enzyme is modulated upon binding of said binding molecule to the mimetope;] and

detecting the amount of catalysis of the substrate [achieved by the chimeric enzyme in comparison to the amount of catalysis of the substrate observed in the absence of said chimeric enzyme] and thereby determining the presence or absence of said analyte of interest.

20. (Twice Amended) A method for determining the presence or amount of an analyte in a test sample, comprising:

forming a mixture of [contacting] (1) a chimeric enzyme comprising an enzyme and a mimetope, said mimetope including at least one amino acid, said chimeric enzyme having a sequence of said mimetope inserted in said enzyme or replacing at least one amino acid of enzyme with the proviso that the activity of the chimeric enzyme is modulated upon binding of a binding molecule to the mimetope, [with a] ([1] 2) test sample containing said analyte of interest, and ([2] 3) a substrate upon which the chimeric enzyme catalytically acts[, to form a reaction mixture, wherein said chimeric enzyme comprises a starting enzyme comprising a polypeptide and said mimetope, said mimetope comprising at least one amino acid, wherein said chimeric enzyme is constructed by inserting a sequence of said mimetope into a sequence of said starting enzyme or replacing at least one amino acid of the starting enzyme with a sequence of said mimetope, with the proviso that the enzymatic activity of the chimeric enzyme is equivalent to that of the starting enzyme and the activity of the chimeric enzyme is modulated upon binding of said binding molecule to the mimetope]; and

detecting the amount of catalysis of the substrate [achieved by the chimeric enzyme in comparison to the amount of catalysis of the substrate observed in the absence of said chimeric enzyme] and thereby determining the presence or absence of said analyte of interest.

Please enter the following new claims:

26. (New) The method of claim 13, wherein the mimetope comprises any one of a sequence identified from SEQ ID NOs. 1-78.

27. (New) The method of claim 20, wherein the mimetope comprises any one of a sequence identified from SEQ ID NOs. 1-78.

28. (New) The method of claim 13, wherein the enzymatic activity of the chimeric enzyme in the unbound state is equivalent to that of the starting enzyme.

29. (New) The method of claim 13, wherein the enzymatic activity of the chimeric enzyme in the unbound state is equivalent to that of the starting enzyme.

IN THE SPECIFICATION:

Please delete the Sequence Listing submitted with the January 21, 2000 Amendment in response to the July 27, 1999 Official Action and insert the revised Sequence Listing, herewith submitted, at the end of the subject specification. At pages 30-35, please delete Tables 1-6 and 8-9 and substitute the revised Tables 1-6 and 8-9. The revised tables include the appropriate "SEQ ID NO" identifier for each sequence listed and the corresponding three letter abbreviation for each amino acid listed in the sequences. No new matter is added. Submitted herewith are two computer readable diskettes, copy 1 and 2, containing the Sequence Listing. The diskettes were encoded using the Microsoft Windows operating system and Microsoft Word as the wordprocessor. All previous computer readable copies are to be deleted.

--Table 1: Sequences and activities of lib 1 A clones selected on 10 µg ampicillin/ml at 37°C

Clones	Inserted Sequence			Kcat (s^{-1})[a]
FdBla	Val_{103}	- - - - Glu_{104} Tyr_{105}	Ser_{106}	ND
Lib1A-01		- - - - Val Ser		29
Lib1A-02		- - - Leu His Ser		16
Lib1A-03		Lys Ala Gly Ser Asp Gly (SEQ ID NO: 1)		70
Lib1A-04		Gly Gly Pro Arg Ser Trp (SEQ ID NO: 2)		15
Lib1A-05		Lys Asn Cys Gly Lys Cys (SEQ ID NO: 3)		12
Lib1A-06		Asp Val Pro Gly Ala Gly (SEQ ID NO: 4)		47
Lib1A-07		Lys Ser Gly Glu His Ser (SEQ ID NO: 5)		145
Lib1A-08		- - - Pro Gly Gly		74
Lib1A-09		Arg Ala Gly Asn His Ser (SEQ ID NO: 6)		265
Lib1A-010		Asp Pro Pro Gly Tyr Gly (SEQ ID NO: 7)		9

[a]kcats from phages produced at 23°C (PenG)
ND: not done

Table 2: Sequences and activities of lib1C_4 clones

Clones	Inserted sequence			Kcat (s^{-1})[a]
FdBla	Val_{103}	- - - - Glu_{104} Tyr_{105}	Ser_{106}	ND
LibC4-11		Arg Phe Gly Asn Asp Trp (SEQ ID NO: 8)		159
LibC4-12		- - - - Trp Trp		ND
LibC4-13		- - Arg Ser His Trp (SEQ ID NO: 9)		ND
LibC4-14		- - - - Gln Trp		ND
LibC4-15		Asp Gln Met Gly Gly Gly (SEQ ID NO: 10)		ND
LibC4-16		Arg Ala Gly Ser Thr Trp (SEQ ID NO: 11)		64
LibC4-17		Lys Gly Gly Leu Glu Ser (SEQ ID NO: 12)		721
LibC4-18		- - - - Ser Asn		ND
LibC4-19		- - - - Glu Gly		ND

[a]kcats from phages produced at 23°C (PenG)
ND: not done

Table 3: Sequences and activities of lib1D_2 clones

Clones	Inserted sequence			Kcat (s^{-1})[a]
FdBla	Leu_{102}	- - - Val_{103} Glu_{104} Tyr_{105}	Ser_{106}	ND
Lib1D2-02		- - - Val Gly Gly		ND
Lib1D2-03		- - - Val Thr Tyr		ND
Lib1D2-04	Phe	- - - Gly Thr Trp		ND
Lib1D2-05		Leu Pro Asn Leu Asp Thr (SEQ ID NO: 13)		224
Lib1D2-06		- - - Ile Ser Trp		ND
Lib1D2-07		Asn Arg Ser Gly Ser Trp (SEQ ID NO: 14)		2506
Lib1D2-08		Asp Val Ser Gly Gly His (SEQ ID NO: 15)		337
Lib1D2-09		Leu His Ser Gly Gly Trp (SEQ ID NO: 16)		ND
Lib1D2-10		Ser Arg Ala Gly Gly Tyr (SEQ ID NO: 17)		ND

[a]kcats from phages produced at 23°C (PenG)
ND: not done

Table 4: Sequences and activities of several clones from the lib3d library picked from among the 3% most active ones

Clones	Inserted sequence			Kcat (s^{-1})[a]
FdBla	Ala_{270}	- - - Thr_{271} Met_{272}	Asp_{273} Glu_{274} Arg_{275}	ND
Lib3-01		- - - Ser Met		1133
Lib3-02		- - Ala Thr Thr		203
Lib3-03		Thr Ala Lys Met Asp (SEQ ID NO: 18)		127
Lib3-04	Pro	Pro Thr Val Ser Met (SEQ ID NO: 19)		92
Lib3-05		Arg Gln Ser Thr Met (SEQ ID NO: 20)		48
Lib3-06	Asp	- - Asp Arg Ala		1.1
Lib3-07		Gly Arg Thr Thr Met (SEQ ID NO: 21)		44
Lib3-08		Ser Asp Gln Pro Leu (SEQ ID NO: 22)	Leu	140
Lib3-09		His Thr Ala Ser Met (SEQ ID NO: 23)		137
Lib3-10		- - - Asn Gly		278
Lib3-11		Lys Ser Val Gly Leu (SEQ ID NO: 24)		ND
Lib3-12		Ala Asn Ile Ser Leu (SEQ ID NO: 25)		ND
Lib3-13		- - - Asn Ile		ND
Lib3-14		Pro Val Ala Pro Ile (SEQ ID NO: 26)		ND
Lib3-15		Arg Pro Thr Thr Leu (SEQ ID NO: 27)		ND
Lib3-16		Pro Asn Ala Asn Met (SEQ ID NO: 28)		ND
Lib3-17		- - Ala Thr Thr		ND

[a]kcats from phages produced at 23°C (PenG)
ND: not done

Table 5: Sequences and activities of lib3f clones selected on 10 μg ampicillin/ml at 37°C

Clones	Inserted sequence			Kcat (s^{-1})[a]
FdBla	Ala_{270}	- - - - - Thr_{271}	Met_{272} Asp_{273} Glu_{274} Arg_{275} (SEQ ID NO: 40)	ND
Lib3-18		Ala Thr Ser Phe Ala [Phe] Pro (SEQ ID NO: 29)		208
Lib3-19		Arg Arg Lys Gln Pro Thr (SEQ ID NO: 30)		32
Lib3-20		Thr Ala His Val Ala Ser (SEQ ID NO: 31)		99
Lib3-21		Thr Asn Lys Gln Pro Ser (SEQ ID NO: 32)		73
Lib3-22		Lys Ser Tyr Thr Pro Glu (SEQ ID NO: 33)	Gln	85
Lib3-23		Lys Trp Asn Tyr Thr Thr (SEQ ID NO: 34)		ND
Lib3-24		Gly Glu His Glu Ala Gly (SEQ ID NO: 35)		114
Lib3-25		Glu Glu Asn Gly Arg Pro (SEQ ID NO: 36)	Gln	100
Lib3-26		Gln Leu Gln Val Pro Pro (SEQ ID NO: 37)		186
Lib3-27		Ala Pro Gly Asn Asp Gly (SEQ ID NO: 38)		64
Lib3-29		Ala Gly Ala Thr Tyr Glu (SEQ ID NO: 39)		111

[a]kcats from phages produced at 23°C (PenG)
ND: not done

Table 6: Sequences and activities of rec 1 clones selected on 10 µg ampicillin/ml at 37°C

Clones	Inserted Sequence							Kcat(s^{-1})[a]
FdBla	Leu_{102}	- - - Val_{103} Glu_{104} Tyr_{105}	Ser_{106}	- - -	Ala_{270}	- - - - - Thr_{271}	Met_{272}	ND
Rec 1-01		Glu Arg Ser Gly His Trp (SEQ ID NO: 41)				- - - - - Thr		145
Rec 1-03		- - - Val Glu Tyr				Arg Thr Ala Lys Val Ser (SEQ ID NO: 44)		57
Rec 1-04		- - - Val Thr Trp				Gln Lys Val Glu Pro Ser (SEQ ID NO: 45)		61
Rec 1-05		- - - Val Leu Gly				- - - - - His		145
Rec 1-06		- - - Val Gln Gly				Thr Gly Val Tyr Pro Ser (SEQ ID NO: 46)		170
Rec 1-07		- - - Cys Met Gly				Gln Gly Pro Trp Ala Ser (SEQ ID NO: 47)		380
Rec 1-09*		- - - Ile Glu Gly				Ile Gly Asp Tyr Ser Lys (SEQ ID NO: 48)		251
Rec 1-10		- - - Val Asp Trp				Thr Gly Asn Gln Ala Thr (SEQ ID NO: 49)		93
Rec 1-11*		- - - Val Ser Gly				Ser Asn Gly Glu His Ser (SEQ ID NO: 50)		54
Rec 1-12		-Leu Ala Ser Gly Tyr (SEQ ID NO: 42)				Ser Gly His Glu Pro Thr (SEQ ID NO: 51)		139
Rec 1-14		- - - Val Pro Tyr				Asp Ser Lys Glu Thr Ser (SEQ ID NO: 52)		304
Rec 1-15*		Val Arg Ser Gly Pro Trp (SEQ ID NO: 43)				Thr Ala Arg Trp Ala Asn (SEQ ID NO: 53)		72
Rec 1-16		- - - Val Met Gly				Thr Ala Asn Glu His Thr (SEQ ID NO: 54)		155

[a]kcats from phages produced at 23°C (PenG)

ND: not done

*clones containing an additional mutation (Arg_{275}^{L})

Table 8: Clones selected on psa 10.

Clones	Inserted Sequences		Kcat-psa66/+psa66 (s^{-1})*
			S=PenG
FdBla	Val_{103} Glu Tyr	Thr_{271} Met	
			[psa10]=3.3 10^{-7}M
P10Aj3	Library[a]		187/179
P10Aj301	Val Glu Tyr	His Pro Gln Asn Asp Asp Met (SEQ ID NO: [58] 59)	ND
P10Aj302	Val Glu Tyr	His Pro Gln Asn Asp Asp Met (SEQ ID NO: 60)	ND
P10Aj303	Val Glu Tyr	His Pro Gln Asn Asp Asp Met (SEQ ID NO: 61)	ND
P10Aj304	Val Glu Tyr	His Pro Gln Gly Asp Asn Met (SEQ ID NO: 62) His Pro Gln Gly Asp Ser Met (SEQ ID NO: 63)	ND
P10Aj305	Val Glu Tyr	His Pro Gln Asn Asp Asp Met (SEQ ID NO: 64)	ND
			[psa10]=3.3 10^{-7}M
P10RB3	Library[b]		52/52
P10RB311	Val Arg Tyr	Ser Asp Gly His Arg Leu Met (Arg_{275}→ Leu) (SEQ ID NO: 65)	ND
P10RB312	Val Lys Ser Gly Val Ala (SEQ ID NO: 55)	Ser Asp Gly His Arg Leu Met (Arg_{275}→ Leu) (SEQ ID NO: 66)	ND
P10RB313	Val Lys Ser Gly Asn Thr Trp (SEQ ID NO: 56)	Ser Asp Gly His Arg Leu Met (Arg_{275}→ Leu) (SEQ ID NO: 67)	ND
P10RB314	Val Asp Arg Thr Lys Gly Trp (SEQ ID NO: 57)	Ser Asp Gly His Arg Leu Met (Arg_{275}→ Leu) (SEQ ID NO: 68)	ND
P10RB315	Val Asp Gly Pro Asn Gly His (SEQ ID NO: 58)	Ser Asp Gly His Arg Leu Met (Arg_{275}→ Leu) (SEQ ID NO: 69)	ND

[a]lib3j and [b]rec[4b] phages from the third round of selection

*kcats from phages produced at 23°C

Table 9: Clones selected on psa66.

Clones	Inserted Sequence		Kcat-psa66/+psa66(s^{-1})*; %age inhibition				
			S=PenG	S=PADAC		S=Centa	
FdBla	Val_{103} Glu Tyr	Thr_{271} Met					
			[psa66]=3.3 10^{-7}M	[psa66]=3.3 10^{-7}M			
P66Aj3	Library[a]		444/425; 04%	ND			
P66Aj306	Val Glu Tyr	Thr Pro Gly Ser Leu Gln Met (Arg_{275}→ Leu) (SEQ ID NO: [70] 71)	ND	67.9/65.8; 03%			
P66Aj307	Val Glu Tyr	Ser Ala His Gln Asp Tyr Ile (Arg_{275}→ Leu) (SEQ ID NO:[71] 72)	ND	42.4/42.4; 00%			
P66Aj308	Val Glu Tyr	Thr Pro Gly Ser Leu Gln Met (Arg_{275}→ Leu) (SEQ ID NO: [72] 73)	ND	ND			
P66Aj309	Val Glu Tyr	Thr Pro Gly Ser Leu Gln Met (Arg_{275}→ Leu) (SEQ ID NO:[73] 74)	ND	ND			
P66Aj310	Val Glu Tyr	Thr Pro Gly Ser Leu Gln Met (Arg_{275}→ Leu) (SEQ ID NO:[74] 75)	ND	ND			
			[psa66]=3.3 10^{-7}M	[psa66]=3.3 10^{-7}M	[psa66]=1.7 10^{-6}M	[psa66]=3.3 10^{-7}M	[psa66]=1.7 10^{-6}M
P66RB3	Library[b]		405/326; 20%	23.8/14.2; 41%	ND	12.2/6.7; 45%	ND
P66RB316	Val Lys Gly	Asp Gly Ser Arg Ile Gln Met (Arg_{275}→ Leu) (SEQ ID NO:[75] 76)	182/134; 26%	25.1/[13/6]13.6; 46%	20.5/7.8; 62%	14.7/7.2; 51%	15.4/4.1; 73%
P66RB317	Val Lys Gly Gly	Thr Leu	ND	28.2/26.5; 06%	ND	ND	ND

	His Gly Ala (SEQ ID NO: 70)						
P66RB318	Val Val Gly	Asp Gly Ser Arg Ile Gln Met (Arg_{275}→ Leu) (SEQ ID NO:[76] 77)	ND	28.6/11.9; 58%	ND	13.8/5.8; 58%	13.3/3.5; 74%
P66RB319	Val Gln Gly	Asp Gly Ser Arg Ile Gln Met (Arg_{275}→ Leu) (SEQ ID NO: [77] 78)	ND	47.4/32.6; 31%	ND	ND	ND
P66RB321	ND	ND	ND	17.2/09.3; 46%	ND	ND	ND
P66RB322	ND	ND	ND	27.2/23.8; 13%	ND	ND	ND
P66RB323	ND	ND	ND	19.0/13.2; 31%	ND	ND	ND
P66RB324	ND	ND	ND	22.4/[15/2]15.2; 32%	ND	ND	ND
P66RB325	ND	ND	ND	21.6/14.9; 31%	ND	ND	ND
P66RB326	ND	ND	ND	19.6/19.2; 02%	ND	ND	ND
P66RB327	ND	ND	ND	20.5/19.6; 04%	ND	ND	ND
P66RB328	ND	ND	ND	29.2/15.8; 46%	ND	ND	ND
P66RB329	ND	ND	ND	26.3/14.3; 46%	ND	ND	ND
P66RB330	ND	ND	6015/4273; 29%	647/444; 31%	ND	33.5/46.2; -32%	33.2/[54.71]53.7;-62%
P66RB331	ND	ND	ND	25.7/14.1; 45%	ND	ND	ND
P66RB332	ND	ND	ND	25.2/23.5; 09%	ND	ND	ND

[a]lib3j and [b]rec4b phages from third round of selection
*kcats from phages produced at 23°C--.

REMARKS

Favorable reconsideration and allowance are respectfully requested. Claims 13-25 are pending in this application.

By this amendment, claims 13 and 20 have been amended and claims 26-29 have been added to further define the invention.. No new matter has been added. Accordingly, upon entry of this amendment, claims 13-29 are pending in this application.

No additional fee is believed necessary for entry and consideration of the enclosed claims.

The revised tables and Sequence Listing were necessitated by the discovery of some inaccuracies. For example, in tables 3 and 4 some single amino acid sequences were inadvertently omitted, table 8 had two different amino acid sequences with the same SEQ ID NOs and in table 9 the amino acid sequence was specified but the "SEQ ID NO: 70" was omitted. No new matter is added. Original tables 3 and 4 of the specification contain the single amino acid sequences now in the revised tables 3 and 4 submitted herewith. Further submitted herewith is a paper Sequence Listing and two diskettes in computer readable form containing copies of the Sequence Listing. Applicants submit that the contents of the paper Sequence Listing and computer readable copies are the same and do not include new matter.

Applicants respectfully submit that the finality of the Office Action mailed March 15, 2000 is improper. The Examiner stated that the Applicants' amendments necessitated the new grounds of rejection and made the Office Action final. Office Action, ¶ 9. Applicants respectfully submit that it was improper to issue a final office action which included a new ground of rejection and hence request that the finality of the Office Action be withdrawn.

Specifically, an office action containing a new ground of rejection may not be final unless that rejection was necessitated by the Applicants' amendment:

> Under present practice, second or any subsequent actions on the merit shall be final, except where the examiner introduces a new ground of rejection that is neither necessitated by applicant's amendment of the claims nor...

MPEP § 706.07(a). The finality of the office action here is improper because the Examiner's "new" grounds of rejection was issued with respect to claim limitations which were present in the previous pending claims confirming that the Applicants' amendment did not cause the new ground of rejection.

More specifically, in the Final Office Action mailed March 15, 2000, the Examiner issued a new rejection of claims 13-25 under 35 U.S.C. § 112, second paragraph, because the claims "recite the phrases 'inserting a sequence...or sequence of said starting enzyme...' and 'with a sequence of said mimetope...". More specifically, the Examiner argued: "It is unclear what sequence(s) are being referred to as no SEQ ID NOs pertaining to the claimed recitation is identified or is apparent" (Official Action, page 2). Applicants submit that this new rejection was not necessitated by amendment. In the previous Amendment filed January 24, 2000, claim 13 was amended to be placed in independent form by incorporating the limitations of claim 1 and claim 1 was cancelled and the specific term objected to by the Examiner, "sequence", was previously pending in dependent claim 10:

> 10. A chimeric enzyme of claim 1, wherein the mimetope is a random peptide **sequence**.

Thus, the finality of this Office Action is improper since Applicants' amendment did not necessitate the new ground for rejection as the term objected to by the Examiner was present and

before the Examiner in the previously pending claims. Therefore, the Examiner is respectfully requested to withdraw the finality of this Office Action.

1. The Examiner has rejected claims 13-25 under 35 U.S.C. § 112, 2nd paragraph, as allegedly being indefinite. The Examiner states that "claims 13 (lines 8-9) and 20 (lines 7-8) recite the phrases 'inserting a sequence... or sequence of said starting enzyme...' and 'with a sequence of said mimetope...'. It is unclear what sequence(s) are being referred to as no SEQ ID NOs... is identified or is apparent" (Official Action, page 2).

37 C.F.R. §§ 1.821-825 merely requires the appropriate SEQ ID NO identifier for all unbranched "specifically identified" amino acid sequences of four or greater. Furthermore, MPEP 2422.01 sets forth that "Sequences with fewer than four specifically defined nucleotides or amino acids are specifically excluded...".

Applicants respectfully submit that claims 13 and 20 of the present invention are not limited to specifically defined sequences. Sequence ID NOs are only required when claims are limited to a specifically defined sequence, therefore claims 13 and 20 satisfy the requirements of 35 U.S.C. § 112, 2nd paragraph.

Claims 13 and 20 claim a chimeric enzyme comprising a starting enzyme and a mimetope. The mimetope may consist of any sequence(s) and is not limited to the sequences disclosed in the specification. The SEQ ID NOs provided in the application identify specific sequences only as examples. A person of ordinary skill in the art will understand that the term "sequence" in claims 13 and 30 is a general term, which is not limited to any particular sequence.

The following quotation emphasizes the relationship between the SEQ ID NOs and 35 U.S.C. § 112.

> The rules do not alter, in any way, the requirements of 35 U.S.C. § 112. The implementation of the rules has had no effect on disclosure and/or claiming requirements.... The use of sequence identification numbers (SEQ ID NO:X) only provides a shorthand way for applicants to discuss and claim their inventions. These identification numbers do not in any way restrict the manner in which an invention can be claimed. MPEP 2422.03

The Examiner includes claims 14-19 and 21-25 in the rejection under 35 U.S.C. § 112, second paragraph "for failing to correct the defect of base claims".

Applicants hereby submit that the statements made above obviate the Examiner's rejections of claims 14-19 and 21-25.

2. The Examiner rejected claims 13-25 under 35 U.S.C. § 112, first paragraph, stating that "as the disclosure is enabling only for claims limited to a method of determining the amount of an analyte in a test sample using a chimeric β-lactamase as the starting enzyme, and comprising selected amino acids sequence insert(ed) in the loop of the rim of the active site residues 103-105 or the alpha 11 helix residues 271-272 of the R-tem β-lactamase... However the guidance provided for a single specific chimeric β-lactamase is inadequate ... to develop the method using any chimeric enzyme construct for determining the amount of an analyte in the sample" (Official Action, pages 2 and 3).

Applicants respectfully traverse this rejection for reasons set forth below.

The amino acid sequence of an enzyme can be modified by insertion of a mimetope at a location which is preferably remote from the active site of the enzyme. Alternatively, the enzyme can also be modified by replacing one or more amino acids by the mimetope sequence. These modifications will yield a chimeric enzyme with activity equivalent to the original starting enzyme wherein the activity is modulated upon binding of a binding molecule to the mimetope.

The specification on pages 2-9 provides disclosure and guidance in obtaining such a chimeric enzyme.

With the availability of a library of mimetopes and the routine screening methods available to those skilled in the art, specific information about sequence homology or functional similarity among different enzymes to modify a starting enzyme for the desired activity is not required.

The Examiner states on page 6 of the Official Action:

> While it is known that many amino acid substitutions are generally possible in any given protein the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation are limited. Certain positions in the sequence are critical to the protein's structure/function relationship...and in providing the correct three-dimensional spatial orientation of binding and catalytic sites... However, applicants have provided little or no guidance beyond the mere presentation of specific sequence inserts in β-lactamase...

In contrast, the applicants have specifically described employing the three-dimensional structure in one of the techniques that is used for selecting and specifically identifying a desired location on the molecule to be engineered.

As described in the specification on p. 11, a particular embodiment of the invention involves assessing the three-dimensional structure of the enzyme. The three-dimensional structural information is a good predictor of the resultant enzyme activity. This technique was used for the β-lactamase and the method is applicable to a variety of different enzymes. Once the three-dimensional structure of the target molecule is known, a site can be selected by specifically identifying a desired location on the molecule to be engineered. For some purposes, it may be desirable to select an exposed site on the surface of the target molecule, where the site is available for attachment by the binding molecule. The three-dimensional structure can be determined by crystallography and/or deduced from known structures and amino acid sequence

data. There are no genetic engineering or molecular biology constructs that would suggest that such three dimensional structural analysis is only uniquely applicable to the β-lactamase enzyme and to no other.

Applicants, hereby, direct the Examiner to a recent publication by Ferrer-Miralles et al., *J. Biol. Chem.* 276 (43):40087-40095 (2001) which demonstrates a similar approach for making galactosidase chimeras. The authors used an epitope insertion and a binding molecule for modulating the activity of the chimeric enzyme based on a three-dimensional structural analysis model for engineering regulatable enzyme activity. Although, the authors used epitopes the same result can be achieved using mimetopes as shown in Applicant's specification. Hence this recent publication provides additional confirmation that the technique is applicable not only to β-lactamases but also to other types of enzymes. For the Examiner's convenience a copy of the Ferrer-Miralles et al. publication is enclosed.

An alternative approach to three-dimensional structure analysis is to select target molecular sites that are susceptible to limited proteolysis or sites strongly predicted to be loops by secondary structure prediction or by analysis of hydrophobic patterns suitable for insertion or replacement engineering. Another alternative approach is to engineer a binding site moiety at random positions within the target molecule. The engineered site is preferably not at the active site of the enzyme but is more preferably located remotely (e.g. at 1-25 Å). The activity of the chimeric molecule is regulatable by binding to the inserted or replaced sequence, irrespective of whether the modification is close to or remote from the active site.

Target and chimeric molecules are prepared by methods available in the art (specification, p.12-16). Genetic engineering techniques can be employed to prepare target and chimeric molecules comprising amino acids or nucleotide residues. In one embodiment, a

cloned gene is employed as the starting material for the starting target molecule and resultant chimeric target molecule. The cloned gene for the starting enzyme β-lactamase serves as the beginning material to produce the chimeric enzyme. In addition, a binding site moiety can be engineered into the starting target molecule using a replacement vector via homologous recombination. Examples are provided in the specification, p. 12-24, of many different types of embodiments with supporting references from the available art.

The Examiner states in the Official Action on page 7 that "...a modification made in one type of ...β-lactamase having a specific sequence may not necessarily translate to or [be] appropriate to make in another kind of β-lactamase."

The applicants have addressed this concern by providing standard screening procedures which are routinely used in the art to determine the appropriate or desired enzymatic activity. The starting enzyme β-lactamase hydrolyzes a β-lactam bond. Various compounds can be used as substrates, including penicillins, cephalosporins, ampicillin, etc. and with each starting molecule a library of mimetopes is created and the library screened for appropriate biological activity as described in Example 1 of the specification (p. 26). A chimera that retains the activity of the starting enzyme can be identified with only routine screening and without undue experimentation. The level of skill in the art is high and given the extensive guidance that is provided in the specification together with the knowledge generally known by those skilled in the art of genetic engineering and molecular biology a chimeric molecule, using any starting molecule, can be prepared without undue experimentation. Applicants have enabled the general method of preparing a chimeric enzyme and demonstrated this method with a particular species of β-lactamase. Based on the extensive guidance provided in the disclosure, including the examples illustrating the invention, all that is required of the skilled artisan to practice the

invention is to identify a starting material, prepare a library of mimetopes and insert the sequences into the starting material and screen for the activity.

The Examiner cites the MINIREVIEW by Bush et al. in *Antomicrobial Agents & Chemotherapy* 39(6): 1211-1233 (1995) as supporting the conclusion "that there are a diverse classes of β-lactamases including Cephalosporins, Penicillin and β-lactams with differing sequence similarities." (Official Action, p.7).

In the Bush et al. article (p. 1217, coln. 2, 2nd para. lines 5-7) the authors state that "...β-lactamases are known to encompass a great deal of diversity in the number of amino acid substitutions that can be tolerated with the retention of β-lactam-hydrolyzing activity..." It is the structural similarities of the β-lactamases that retains the enzyme's functional activity despite differing sequences. Applicants maintain that the invention is not limited to β-lactamases but is generally applicable to any enzyme. Guidance and direction is provided in the specification to determine the structure of the enzyme and the disclosure provides the appropriate screening methods for generating a library of mimetopes. Therefore focusing only on the differing enzyme sequences is largely irrelevant.

Enablement is not precluded by the necessity for experimentation such as routine screening. In re Wands, 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988). The following case law and MPEP citations further explains the extent to which experimentation is permitted.

> The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands*, 858 F.2d 731, 737, 8 U.S.P.Q. 2d 1400, 1404 (Fed. Cir. 1988) and MPEP 2164.06.
>
> The test for enablement is whether one reasonably skilled in the art to make or use the invention from the disclosure in the patent coupled with information known in the art without undue experimentation. A patent may be enabling even though some

> experimentation is necessary. *United States v. Telectronics, Inc.*, 857 F.2d 778, 8 U.S.P.Q. 2d 1217 (Fed. Cir. 1988).
>
> The enablement requirement is met if the patent application enables "any mode" of making and using the claimed invention. *William Service Group, Inc. v. O.B. Cannon & Son, Inc.*, 33 U.S.P.Q. 2d 1705, 1723 (Eastern District of Pennsylvania 1994). A patent application may be enabling even though some experimentation is required, but the amounts of experimentation must be reasonable. Id.
>
> An extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. *In re Colianni*, 561 F.2d 220, 224, 195 U.S.P.Q. 150, 153 (C.C.P.A. 1977), MPEP 2164.06.

Therefore, it is respectfully submitted that one of ordinary skill in the art would only require routine experimentation to practice the disclosed invention.

3. The Examiner rejected claims 13-14, 16-25 under 35 U.S.C. § 102(b) as being anticipated by Rodrigues et al. (Cancer Research 55:63-70, 1995).

Applicants respectfully traverse the 35 U.S.C. § 102(b) rejection. To anticipate a claim a reference must contain all of the elements of the claim. *Hybritech, Inc.* v. *Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379 (Fed. Cir. 1986); *In re Marshall*, 578 F.2d 301 (C.C.P.A. 1978).

The Rodrigues article describes the use of β-lactamase as an agent for use in targeted prodrug therapy. The reference describes attaching a portion of an antibody, the binding molecule, to the β-lactamase. The enzyme can then be attached at the site of the tumor cell of interest through the binding molecule linked to this enzyme. The prodrug circulating near this tumor cell and antibody-enzyme complex is then activated and predominantly exerts its toxic effect on the tumor cell.

Once the Prodrug comes into contact with the β-lactamase enzyme the prodrug is cleaved and its toxic effect is achieved. The β-lactamase enzyme would have had the same substrate activity before attachment to the antibody. The only difference is that Rodrigues designed the cleavage to occur near the site of the tumor cells. The binding molecule, the antibody, does not change or modulate the enzymatic activity level of β-lactamase. This was verified in Rodrigues, p.67, Table 2, the substrate activity is similar for both β-lactamase and dsF_V3-β-lactamase.

Therefore the Rodrigues binding molecule is not a mimetope as defined in the present invention. There is no disclosure or suggestion in Rodrigues that the catalytic activity of the β-lactamase enzyme is modulated. Merely attaching a binding molecule to the enzyme does not automatically affect the catalytic activity of that enzyme as the Examiner's statement (Official Action, p. 10, section (4)). Changing the location of the site of action of the enzyme (to the tumor cell of interest) does not mean that the enzyme's catalytic activity is modulated where the modulation is defined in the invention as the actual substrate activity of the enzyme.

The Rodrigues fusion is only designed to attach the β-lactamase enzyme to the antibody and hence to a specific cell surface. The antibody which is directly fused to the β-lactamase does not modulate the enzymatic properties of the enzyme nor was it designed for such purposes. In contrast, the invention specifically discloses the modulatory nature of the binding molecule on the substrate activity of the β-lactamase enzyme (p. 10-11 of the specification). The Rodrigues document does not disclose that the enzyme activity is regulatable nor is the chimeric enzyme used for detecting the amount of substrate catalysis achieved. Therefore the Rodrigues article does not contain all of the elements of claims 13-14 and 16-25 and hence it does not anticipate.

To summarize, Rodrigues article does not suggest that the binding molecule controls the

modulatory behavior of the enzyme for the regulation of substrate activity and therefore does not render the invention obvious.

Favorable reconsideration of this application is respectfully requested.

Respectfully submitted,

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